REMARKS

This Reply is responsive to the Office Action dated June 20, 2002. Entry of the foregoing and reconsideration on the merits pursuant to 37 CFR 1.112 is respectfully requested.

The claims are amended to recite that the donor cell or cell nucleus and the recipient oocyte are of different ungulate species, and claims 11-15, 25, 28-34, 38, 57 and 58 are amended to specify that the donor cell or cell nucleus and/or the recipient oocyte are of bovine species. Support for the invention wherein the donor and the recipient cells are of different ungulate species is found in the specification, for example, at page 20, lines 3-10. Product claims 27-34 and 56-58 claims are amended to specify that the product cells have mitochondria of the recipient oocyte (the "second ungulate species"), support for which is found in the specification; for example, at page 18, lines 6-13. Claim 50 is amended to recite a detectable marker, the expression of which is operably linked to a promoter that regulates expression of a particular cyclin in an ungulate cell, in accord with the disclosures on pages 20 and 49. Claims 24 and 25 are amended by deleting "or" from between "embryonic" "stem-like," in accord with the previous amendments, and claims 18 and 19 are amended to correct grammatical errors. No new matter was added.

Regarding the objection to the specification:

The first paragraph of the specification incorrectly identified a related application as U.S. Application No. 09/032,995 instead of 09/032,945. The specification is amended to correct this error; and withdrawal of the objection to the specification is respectfully requested.

Regarding the rejection of claims under 35 U.S.C. §112, first paragraph:

Claims 1-34 and 36-58 were rejected under 35 U.S.C. §112, first paragraph, because the specification does not provide enablement, since the claims recite cross-species nucelar transplantation, but the bovine example demonstrating transfer of a somatic gaur cell nucleus into an enucleated cow oocyte, with successful production of a viable calf having gaur genomic DNA, is not supportive, since the gaur and the cow "are both from the same species *Bos*." (Page 5 of the office action). The office action further asserts that true cross-species

nuclear transplantation is expected to produce an embryo that undergoes developmental arrest, and so would not reasonably be expected to produce a blastocyst that could give rise to pluripotent embryonic stem-like cells as described in the present application. The office action cites Meirelles et al. and Dominko et al. in support of this view. The office action concludes that the claimed method is only enabled for nuclear transplantation wherein the donor and recipient cell are subspecies of the same species. The applicants respectfully traverse these grounds of rejection

The Applicants respectfully submit that *Bos taurus* and *Bos gaurus* are distinct, different species within the genus *Bos*. The clearest evidence of this is found in comparison of the chromosomes of the two species - as shown in the figure attached as Appendix 2a, a normal, diploid cell of a gaur (*Bos gaurus*) has 58 chromosomes, whereas a normal dploid cow cell (*Bos taurus*) has 60 chromosomes. The fetal and newborn calves produced by transfer of a somatic gaur cell nucleus into an enucleated cow oocyte had the chromosome number of the donor gaur cell (58 chromosomes). The National Center for Biotechnological Information of the National Library of Medicine also recognizes *Bos taurus* and *Bos gaurus* as different species within the genus *Bos* (see Appendix 2b). The examiner's arguments that the claims are not enabled because the claimed method would not operate successfully with a donor and recipient of two different species is therefore without support. In view of the foregoing, withdrawal of the enablement rejection under 35 U.S.C. §112, first paragraph, is respectfully requested.

Regarding rejection of the claims under 35 U.S.C. §112, second paragraph:

Claims 50-54 were rejected under the second paragraph of 35 U.S.C. §112, as being indefinite, because the nature of the linkage of expression of the marker to expression of a cyclin is unclear. Claim 50 is amended to recite that the detectable marker is operably linked to a cyclin promoter, as suggested by the examiner. Withdrawal of the rejection under 35 U.S.C. §112, second paragraph, is respectfully requested.

Claims 1-34, 36-49, and 55-58 were rejected under the second paragraph of 35 U.S.C. §112, as being indefinite, because the claims do not specify that the method resluts in production of a nuclear transfer unit having nuclear DNA of one species and mitochondria of a second species. The Applicants respectfully traverse this ground of rejection. The method of the invention is set forth in claim 1 and its dependent claims in clear and precise terms, and

accords with the written description of the invention provided in the specification. The claimed method encompasses nuclear transfer without supplemental transfer of cytoplasm or mitochondria into the recipient oocyte. This was the procedure followed in the transfer of a gaur nucleus into a cow oocyte. The claimed method also encompasses supplemental transfer of cytoplasm or mitochondria into the recipient oocyte, to improve ethe efficiency of reprogramming and viability of the nuclear transfer embryo. The claims clearly set forth the method in definite terms that encompass these embodiments. Accordingly, withdrawal of the rejection of the claims under 35 U.S.C. §112, second paragraph, as being indefinite is respectfully requested.

Regarding rejection of the claims under 35 U.S.C. §102:

Claims 27-32 were rejected under 35 U.S.C. §102(a) as being allegedly anticipated by Granerus et al. The Applicants respectfully traverse this rejection, as the cells of the present claims, having a nucleus of one ungulate or bovine species and mitochondria of a different ungulate or bovine species, as recited in claims 27-32, is not disclosed by Granerus et al. Withdrawal of the rejection is respectfully requested.

Claims 27-34 and 50-54 were rejected under 35 U.S.C. §102(e) as being allegedly anticipated by Tsukamoto et al. Again, the Applicants respectfully traverse this rejection, as Tsukamoto et al does not disclose the cells having a nucleus of one ungulate or bovine species and mitochondria of a different ungulate or bovine species, as recited in claims 27-34, nor does it disclose the ungulate cells of claims 50-54. Withdrawal of the rejection is respectfully requested.

Claims 27-34 and 50-54 were rejected under 35 U.S.C. §102(b) as being allegedly anticipated by Yamane et al. The Applicants respectfully traverse this rejection, again, because Yamane et al. does not disclose the cells having a nucleus of one ungulate or bovine species and mitochondria of a different ungulate or bovine species, as recited in claims 27-34, nor does it disclose the ungulate cells of claims 50-54. Withdrawal of the rejection is respectfully requested.

Regarding rejection of the claims under 35 U.S.C. §103(a):

Claims 27-34, 36 and 50-54 were rejected under 35 U.S.C. §103(a) as being allegedly unpatentable over Tsukamoto et al., on the grounds that it would have been obvious to modify differentiated human cells produced from stem cells as described by Tsukamoto et al. to arrive at the genetically modified cells as claimed in the present claims. The Applicants respectfully traverse this rejection, because Tsukamoto et al. neither discloses nor suggests making or using the cells having a nucleus of one ungulate or bovine species and mitochondria of a different ungulate or bovine species, as recited in claims 27-34, or the ungulate cells of claims 50-54. Withdrawal of the rejection is respectfully requested.

All issues raised by the Office Action dated June 20, 2002, having been addressed in this Reply, a Notice of Allowance is next in order. If the Examiner has any further questions or issues to raise regarding the subject application, it is respectfully requested that the undersigned be contacted so that such issues may be addressed expeditiously.

Respectfully submitted,

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APPENDIX

IN THE SPECIFICATION:

The paragraph starting at line 10 of page 1 is replaced with the following:

-- This application claims priority under 35 U.S.C. §119 to PCT/US99/04608, filed on March 2, 1999. Also, this application is a continuation-in-part of U.S. Serial No. 09/395,368, filed September 14, 1999, which is a continuation-in-part of U.S. Serial No. 09/260,468, filed March 2, 1999, which is a continuation-in-part of application Serial No. [09/032,995] 09/032,945, filed March 2, 1998, which is in turn a continuation-in-part of application Serial No. 08/699,040, filed August 19, 1996. All of these applications are incorporated by reference in their entirety herein.--

IN THE CLAIMS:

Claims 1, 2, 5-8, 10-16, 18-19, 24, 25, 27-34, 38, 50, and 56-58 are amended as shown below:

1. (Twice Amended) A method of producing ungulate embryonic stem-like cells, wherein said cells comprise a nucleus derived from an adult differentiated cell of a first ungulate species and mitochondria from an oocyte of a second ungulate species other than the species of said adult differentiated cell, comprising the following steps:

(v) inserting a donor differentiated [human or mammalian] cell or cell nucleus of said first ungulate species into a recipient animal oocyte of said second ungulate species[, wherein such oocyte is derived from a different animal species than the human or mammalian cell] under conditions suitable for the formation of a nuclear transfer (NT) unit, wherein the endogenous oocyte nucleus is removed or inactivated before, concurrent, or after introduction of donor cell or nucleus;

(vi) activating the resultant nuclear transfer unit;

(vii) additionally inserting into said oocyte cytoplasm derived from [an] a second oocyte or a blastomere of the same species as the donor cell or nucleus;

(viii) culturing said activated nuclear transfer unit until greater than the 2-cell developmental stage;

(v) disassociating said activated nuclear transfer unit; and

(vi) isolating cells from said disassociated nuclear transfer unit to obtain embryonic stem-like cells.

5. (Amended) The method of Claim 4, wherein said second oocyte is an immature oocyte

6. (Amended) The method of Claim 5, wherein said second oocyte is an immature [human] bovine oocyte.

7. (Amended) The method of Claim 5, wherein said immature [human] oocyte is matured *in vitro* prior to isolation of cytoplasm therefrom.

8. (Amended) The method of Claim [4] 5, wherein said immature oocyte is activated *in vitro* prior to isolation of cytoplasm therefrom.

10. (Amended) The method of Claim 2, wherein all or part of the cytoplasm of the recipient oocyte is removed prior to introduction of cytoplasm from said at least one second oocyte or blastomere of the same species as the donor cell or nucleus.

11. (Amended) The method of Claim 1, wherein the cell or cell nucleus inserted into the enucleated [animal] oocyte is a [human] bovine cell.

12. (Amended) The method of Claim 11, wherein said [human] bovine cell is an adult cell.

13. (Amended) The method of Claim 11, wherein said [human] bovine cell is an epithelial cell, keratinocyte, lymphocyte or fibroblast.

14. (Amended) The method of Claim 11, wherein the recipient [oocytes are] oocyte is obtained from a bovine mammal.

15. (Amended) The method of Claim 14, wherein the animal oocyte is obtained from [an ungulate] Bos taurus.

16. (Amended) The method of Claim [15] 1, wherein said first and second ungulate species are both of an ungulate that is selected from the group consisting of bovine, ovine, porcine, equine, caprine, and buffalo.

18. (Amended) The method of Claim 1, wherein the fused nuclear transfer [units are] unit is activated *in vitro*.

19. (Amended) The method of Claim 1, wherein the activated nuclear transfer [units are] unit is cultured on a feeder layer culture.

24. (Amended) The method of Claim 1, wherein the resultant embryonic [or] stem-like cells are induced to differentiate.

25. (Amended) The method of Claim 11, wherein the resultant bovine embryonic [or] stem-like cells are induced to differentiate.

27. (Amended) [Human] Ungulate embryonic stem-like cells obtained according to the method of Claim 1, which cells have mitochondria of said second ungulate species.

28. (Amended) [Human] Bovine embryonic stem-like cells obtained according to the method of Claim 11, which cells have mitochondria of said second ungulate species.

29. (Twice amended) [Human] Bovine embryonic stem-like cells obtained according to the method of Claim 12, which cells have mitochondria of said second ungulate species.

30. (Twice amended) [Human] Bovine embryonic stem-like cells obtained according to the method of Claim 13, which cells have mitochondria of said second ungulate species.

31. (Twice amended) [Human] Bovine embryonic stem-like cells obtained according to the method of Claim 14, which cells have mitochondria of said second ungulate species.

32. (Twice amended) [Human] Bovine embryonic stem-like cells obtained according to the method of Claim 15, which cells have mitochondria of said second ungulate species.

33. (Amended) Differentiated [human] bovine cells obtained by the method of Claim 25, which cells have mitochondria of said second ungulate species.

34. (Amended) The differentiated [human] bovine cells of Claim 33, which are selected from the group consisting of neural cells, hematopoietic cells, pancreatic cells, muscle cells, cartilage cells, urinary cells, liver cells, spleen cells, reproductive cells, skin cells, intestinal cells, and stomach cells, which cells have mitochondria of said second ungulate species.

38. (Amended) The method of Claim 37, wherein said embryonic [or] stem-like cells are [human] bovine embryonic [or] stem-like cells.

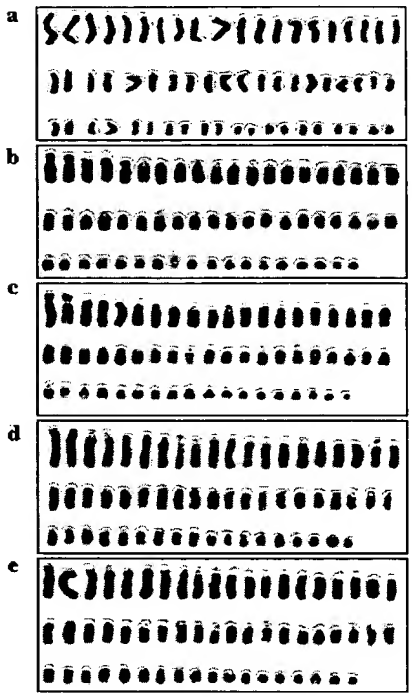
50. (Twice amended) An [mammalian] ungulate somatic cell that expresses a DNA that encodes a detectable marker, the expression of which is operably linked to a promoter that regulates the expression of a particular cyclin.

56. (Amended) An ungulate embryonic stem-like cell isolated from the inner-most portion of a nuclear transfer unit according to the method of claim 55, which cell has mitochondria of said second ungulate species.

57. (Amended) Differentiated [human] bovine cells obtained by the method of Claim [36] 33, wherein said differentiated cells contain and express [and] an inserted gene.

58. (Amended) The differentiated [human] bovine cells of Claim 33, which are selected from the group consisting of neural cells, hematopoietic cells, pancreatic cells, muscle cells, cartilage cells, urinary cells, liver cells, spleen cells, reproductive cells, skin cells, intestinal cells, and stomach cells.--

APPENDIX 2a



Complete chromosome sets of standard domestic bull (*Bos taurus*) and gaur (*Bos gaurus*) that shows that the cloned fetuses are in fact gaurs.

a: The standard domestic bull (*B. taurus*) has a normal diploid chromosome number of 60.

b: The male gaur (*B. gaurus*) has a normal diploid chromosome number of 58.

c-e: The cloned fetuses have a diploid chromosome number of 58.

- From Advanced Cell Technology Media Archive (visited 12/18/02):
http://www.nrp-euro.com/imedia/ikit/act2/html/medarch.html

APPENDIX 2b

Excerpts from the Taxonomy site of the NCBI (National Center for Biotechnological Information) – Bos page - From:
http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=9903 (visited 12/18/02)

Bos

Taxonomy ID: 9903
Genbank common name: **oxen, cattle**
Rank: genus
Genetic code: Translation table 1 (Standard)
Mitochondrial genetic code: Translation table 2

Lineage(abbreviated)

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Cetartiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae

Bos gaurus

Taxonomy ID: 9904
Genbank common name: **gaur**
Rank: species
Genetic code: Translation table 1 (Standard)
Mitochondrial genetic code: Translation table 2

Other names:

seladang[common name]

Lineage(abbreviated)

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Cetartiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos

Bos taurus

Taxonomy ID: 9913
Genbank common name: **cow**
Rank: species
Genetic code: Translation table 1 (Standard)
Mitochondrial genetic code: Translation table 2

Other names:

Bos bovis[synonym], **Bos primigenius taurus**[synonym], **bovine**[common name], **domestic cow**[common name], **domestic cattle**[common name], **cattle**[common name]

Lineage(abbreviated)

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Cetartiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos